

AFRL-RH-BR-TR-2009-0068

Review of Literature on High Power Microwave Pulse Biological Effects

Ronald L. Seaman General Dynamics Advanced Information Systems 8262 Hawks Road Brooks City-Base, TX 78235

August 2009

Interim Report for December 2008 to January 2009

Approved for public release; distribution unlimited. Public Affairs Case file no. 10-002, 6 January 2010; Brooks City-Base, Texas.

Air Force Research Laboratory 711 Human Performance Wing Human Effectiveness Directorate Directed Energy Bioeffects Division Radio Frequency Radiation Branch Brooks City-Base, TX 78235-5147

NOTICE AND SIGNATURE PAGE

Using Government drawings, specifications, or other data included in this document for any purpose other than Government procurement does not in any way obligate the U.S. Government. The fact that the Government formulated or supplied the drawings, specifications, or other data does not license the holder or any other person or corporation; or convey any rights or permission to manufacture, use, or sell any patented invention that may relate to them.

This report was cleared for public release by the 88th ABW Public Affairs Office and is available to the general public, including foreign nationals. Copies may be obtained from the Defense Technical Information Center (DTIC) (http://www.dtic.mil).

AFRL-RH-BR-TR-2009-0068 has been reviewed and is approved for publication in accordance with assigned distribution statement.

//SIGNED//

TAMMARA L. ALEXANDER, CAPT, USAF Contract Monitor Radio Frequency Radiation Branch

//SIGNED//

GARRETT D. POLHAMUS, DR-IV, DAF Chief, Directed Energy Bioeffects Division Air Force Research Laboratory 711 Human Performance Wing Human Effectiveness Directorate

This report is published in the interest of scientific and technical information exchange, and its publication does not constitute the Government's approval or disapproval of its ideas or findings.

Form Approved REPORT DOCUMENTATION PAGE OMB No. 0704-0188 Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS. 1. REPORT DATE (DD-MM-YYYY) 2. REPORT TYPE 3. DATES COVERED (From - To) 17/08/2009 Interim Technical Report December 1, 2008 – January 14, 2009 4. TITLE AND SUBTITLE 5a. CONTRACT NUMBER FA8650-07-D-6800 **5b. GRANT NUMBER** Review of Literature on High Power Microwave Pulse Biological Effects N/A **5c. PROGRAM ELEMENT NUMBER** 62202F **5d. PROJECT NUMBER** 6. AUTHOR(S) 7757 Seaman, Ronald L. 5e. TASK NUMBER В3 **5f. WORK UNIT NUMBER** 7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) 8. PERFORMING ORGANIZATION REPORT NUMBER Air Force Research Laboratory, 711 Human Performance Wing, N/A Radio Frequency Radiation Branch; General Dynamics Advanced Information Systems 8262 Hawks Road Brooks City-Base, TX 78235-5147 9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) 10. SPONSOR/MONITOR'S ACRONYM(S) Air Force Materiel Command, Air Force Research Laboratory 711 HPW/RHDR

Brooks City-Base, Texas 78235-5147 12. DISTRIBUTION / AVAILABILITY STATEMENT

711 Human Performance Wing, Human Effectiveness Directorate

Directed Energy Bioeffects Division, Radio Frequency Radiation Branch

Distribution A: Approved for public release; distribution unlimited. Public Affairs Case file no. 10-002, 5 Jan 2010.

13. SUPPLEMENTARY NOTES

14. ABSTRACT

8262 Hawks Road

Biological effects of pulsed-modulated microwave radiation published in the open scientific literature and unclassified technical reports are reviewed. The effects of short exposures to unmodulated microwaves are included for their possible relevance to exposure to high peak power exposures.

15. SUBJECT TERMS

Microwave pulse, microwave hearing, microwave incapacitation, microwave stunning, microwave fixation, in vitro, animal behavior

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON Tammara L. Alexander
a. REPORT Unclassified	b. ABSTRACT Unclassified	c. THIS PAGE Unclassified	CAD	2.4	19b. TELEPHONE NUMBER (include area code)
Officiassifica	Officiassified	Officiassifica	SAR	24	,

11. SPONSOR/MONITOR'S REPORT

AFRL-RH-BR-TR-2009-0068

NUMBER(S)

THIS PAGE INTENTIONALLY LEFT BLANK

TABLE OF CONTENTS

	Page
1.0 BACKGROUND	1
2.0 LITERATURE REVIEWED	1
3.0 PERCEPTION OF PULSED MICROWAVE RADIATION	2
 3.1 Microwave Hearing	2
4.0 MICROWAVE CONVULSIONS AND STUNNING	5
5.0 MICROWAVE FIXATION	5
6.0 STUDIES WITH TEMPO AND RELATED DEVICES	6
7.0 MICROWAVE PULSES STUDIED USING ISOLATED TISSUES	8
8.0 SUMMARY	8
REFERENCES	11
LIST OF TABLES	
	Page
Table 1: Biological effects of high peak power microwave pulses reported in the open	10

LIST OF ACRONYMS

CF Center frequency
CW Continuous wave

FDTD Finite-difference time-domain MRI Magnetic resonance imaging

SA Specific absorption SAR Specific absorption rate

TEMPO Transformer Energized Megavolt Pulsed Output

EXECUTIVE SUMMARY

Biological effects of pulsed-modulated microwave radiation published in the open scientific literature and unclassified technical reports are reviewed. The effects of short exposures to unmodulated microwaves are included for their possible relevance to exposure to high peak power exposures.

1.0 BACKGROUND

Systems radiating pulsed energy at microwave frequencies (300 MHz to 300 GHz) are used for military communication, detection, and countermeasures. Many of these systems produce pulses in patterns to encode communications or to seek information as in radar. Other systems produce single pulses or a few pulses of energy for detection or countermeasures.

Regardless of pulse pattern, the microwaves are said to be pulse-modulated and the resulting signal can be represented in the frequency domain by a relatively narrow band of frequencies centered at the microwave frequency. Thus, each pulse has electromagnetic characteristics similar to microwaves at a constant power level, or continuous wave (CW). An individual pulse is generally thought of as having a rectangular power profile with a duration, or width, of a few nanoseconds to a few microseconds, and a peak power. Although pulses are actually trapezoidal, they can be described fairly accurately in terms of peak power and width of equivalent rectangular pulses. The rate of energy deposition in tissue is characterized by specific absorption rate (SAR) in W/kg. The absorbed energy is described by specific absorption (SA) in J/kg.

2.0 LITERATURE REVIEWED

The literature on the effects of single microwave pulses on animals and humans is reviewed. Literature on cellular effects of single microwave pulses, mostly from isolated tissue preparations, is also included. In addition, literature on studies using acute exposures, 6 min or shorter, and very high power pulses for longer periods but at low repetition frequencies are covered for perspective. Many of these topics are covered in reviews of the biological effects of microwave and other types of pulses (Lu & de Lorge, 2000; Pakhomov & Murphy, 2000; Heynick, 2003).

Studies on the effects of mobile phone emissions are not covered. Although mobile phone signals use microwave pulses to encode information, the emission levels are low relative to those used in most military applications and exposures during typical use and in experimental investigations can last from many minutes to hours. In addition, research with mobile phones has become rather extensive and, in many cases, yields results that are weak and/or controversial.

Research reports in the open literature and unclassified technical reports were the primary sources of information for this unclassified literature review. Abstracts of the annual meeting of the Bioelectromagnetics Society (2002-2008) were also searched for microwave pulse work. Published reviews of microwave biological effects are sometimes referenced as efficient summaries of effects.

3.0 PERCEPTION OF PULSED MICROWAVE RADIATION

3.1 Microwave Hearing

Microwave hearing is the auditory sensation resulting from microwave energy impinging on the head (Chou et al., 1982; Elder & Chou, 2003). Investigation of microwave hearing began in the late 1950's and 1960's in the field (e.g., Frey, 1961, 1962, 1967). More recent studies address exposure to RF pulses experienced in magnetic resonance imaging (MRI). An experimental study has been done on auditory perception with exposure by RF coils used in MRI (Röschmann, 1991). Theoretical studies of induced acoustic waves with MRI exposure have also been reported (Wang & Lin, 2005; Lin & Wang, 2006).

Microwave hearing is an undisputed effect of pulse modulated microwaves (Guy et al., 1975). The incident energy must be pulse modulated and pulse durations of 3-5000 μs and 0.5-700 μs have been studied in human and animal experiments, respectively (Elder & Chou, 2003). Sensation occurs readily with exposure to pulsed microwaves with very small time-averaged power and energy densities. A single pulse has been reported being sensed as an auditory click. With temperature increase caused by each pulse estimated to be only 10⁻⁶-10⁻⁵ °C at perception threshold (Guy et al., 1975; Chou et al., 1982; Lin, 1978, 1990; Elder & Chou, 2003), the effect is clearly not due to gross heating of tissue.

Auditory sensations in humans and responses in the auditory systems of animals in microwave hearing are directly related to characteristics of individual pulses, and to pulse repetition frequency when it is at auditory frequencies. Energy density, the product of incident power density and pulse duration (also called energy fluence), seems to be a defining pulse characteristic in many of the studies. Threshold energy density for human perception is reported as 2.3-40 $\mu J/cm^2$, depending on the study (Elder & Chou, 2003). Thresholds for detection by the auditory systems in a variety of animal preparations are 1.5-1240 $\mu J/cm^2$ fluence and 0.6-180 mJ/kg SA in the head, again depending on the study (Seaman & Lebovitz, 1989; Elder & Chou, 2003). Amplitude of the pressure transient at microwave hearing threshold has been estimated in a finite-difference time-domain (FDTD) model of the human head to be 0.18 Pa for a 20- μ s pulse (Watanabe et al., 2000).

According to Lin [1978, 1989, 1990], the peak acoustic pressure is a function of pulse duration, head size, and, of course, incident power density. For a 918-MHz, 10-µs pulse with 2.183 W/cm² peak power density incident on a spherical head model with a radius of 7 cm, the calculated peak pressure is 0.682 Pa. The relationship of peak pressure with pulse duration is complex, but, for durations of 0.1 µs and shorter, an asymptotic peak value of approximately 0.037 Pa is reached. This is a factor of 18.4 times between pressures for 10-µs pulses and 0.1 µs and shorter pulses. The ratio of thresholds for perception is thus expected to be roughly 20.

3.2 Thermal and Pain Sensations

Because the absorption of microwave energy in biological tissues leads to increased temperature, the temperature sense and the sense of pain caused by high temperature are considered. Sensations of warmth and thermal pain in the skin have been studied extensively

(Chéry-Croze, 1983; Gardner et al., 2000; Lumpkin & Caterina, 2007). Radiant energy from lamps and lasers as well as contact heating are used as stimuli in traditional studies of physiology of these senses (Arendt-Nielsen & Chen, 2003). One can expect that the same heating by a microwave exposure and by other means will result in similar sensations. However, the rate of change of temperature might have to be taken into account (Yarnitsky et al., 1992; Nielsen & Arendt-Nielsen, 1998; Hilz et al., 1999; Defrin et al., 2006).

Skin temperature thresholds for sensations of warmth and thermal pain are reported as 33-40 °C, and 38-46 °C, respectively (Jørum et al., 2003; Al-Saadi et al., 2006; Ørstavik et al., 2006). The rather broad and overlapping ranges of thresholds reflect the influence of several factors on the sensations, including environmental temperature (Strigo et al., 2000), temperature of the skin before application of a thermal stimulus (Arendt-Nielsen & Bjerring, 1988; Nahra & Plaghki, 2005), and location on the body of the stimulus (Ørstavik et al., 2006). Although decreased sensation with age occurs, whether the subject is male or female does not seem to influence the thresholds (Hilz et al., 1999; Pickering et al., 2002; Schaffner et al., 2008).

Microwave radiation is capable of evoking warmth and pain sensations. An early published study looked at the pain threshold for 10-cm (~3 GHz) microwaves using a horn or an open-ended waveguide filled with a dielectric that contacted the skin at different locations on the body (Cook, 1952). Burning pain was first sensed when the increase in skin temperature was 15 °C, and for 30-130-s exposures this corresponded to approximately 0.6 cal-cm⁻²s⁻¹ at short durations down to about 0.24 cal-cm⁻²s⁻¹ at long durations (2.5 to 1 W/cm²). The use, although carefully done, of a metal thermocouple to measure temperature and the unspecified method of calculating microwave intensity in this study make the results somewhat tentative for application to a free-field exposure.

In other early work on the sense of warmth, the forearm was exposed to 10-cm microwaves using open-ended waveguides with different apertures contacting the skin (Vendrik & Vos, 1958). Power densities of roughly 0.7-2.6 W/cm² (most likely power divided by aperture area) caused a subject reaction indicating perception of warmth with a delay of about 1 s. Longer delays were associated with lower powers, about 6 s for 0.3 W/cm². Thresholds were determined for fixed shorter durations of exposure in a later study using a similar arrangement (Eijkman & Vendrik, 1961). For durations of 0.25-2 s, threshold energy was about 8-14.6 J, which corresponded to 32.4-7.3 W, presumably at the microwave source used.

In apparently the first reports of microwave sensations with noncontact delivery of microwaves in the laboratory, thresholds were determined for warmth sensation on the forehead (Hendler et al.,1963; Hendler, 1968). Using 3-cm (~10 GHz) microwaves, thresholds ranged roughly from 24.3 down to 13.8 mW/cm² for durations of 0.5 to 5 s (Hendler et al., 1963). For 10-cm (~3 GHz) microwaves, the thresholds were higher: 14.3 down to 7.6 mW/cm² for durations from 1 to 5 s (Hendler, 1968).

In more recent experiments, thresholds have been determined for microwave exposure of the forearm and back (Justesen et al., 1982; Blick et al., 1997). For exposure of the forearm with 2.45-GHz microwaves for 10 s, threshold for warmth sensation was an average 26.74 mW/cm²

(Justesen et al., 1982). Using 10-s exposures of the back, average thresholds for warmth sensations were determined to be 63.1, 19.5, 19.6 mW/cm² for 2.45, 7.5, and 10 GHz, respectively (Blick et al., 1997).

That increased temperature from microwave exposure can lead to the sensation of pain was reported in some of the earliest work on human perception, but other work reported in the open literature confirms this. Studies of signals from the nerve receptors for pain were done in an animal preparation (McAfee, 1961, 1962). The results showed that the receptors reacted to microwave heating in the same way that they reacted to heating by warm water or resistance-wire thermode. In a more recent study with exposure of the back to millimeter wave radiation at 35 GHz for 3 s, pain threshold was associated with an increase of 9.9 °C in skin temperature (Walters et al., 2000).

The sensation of skin pain reported for exposure to millimeter wave exposure is consistent with the penetration depth of this form of radiation being less than about 0.2 mm (Walters et al., 2000). However, absorption of microwave energy at lower frequencies will be deeper with depth and pattern of absorption depending on microwave frequency and tissue anatomy (Johnson & Guy, 1972; Durney et al., 1986; Lin, 1986). For frequencies lower than millimeter wave frequencies, absorption and consequently temperature increase might not be maximal on the body surface.

The possibility of pain sensed as a result of deeper penetration and heating of microwave energy can be appreciated by referring to just a few of the published papers on muscle, bone, and visceral pain. Experimental high intensity thermal stimulation of 48 °C has been found to induce muscle pain (Graven-Nielsen et al., 2002). Threshold for pain in the lower esophagus has been reported as 48.5-52 °C as studied with a contained circulating fluid (Pedersen et al., 2004a,b). Earlier, in ultrasound hyperthermia, bone pain perceived by patients resulted in treatment termination in many cases (Meyer, 1984; Shimm et al., 1988). The most likely cause was a much higher temperature at the bone-muscle interface than in the muscle being treated (Hynynen & DeYoung, 1988).

3.3 Startle Modification and Evoked Motion

Single microwave pulses impinging on the heads of rats placed in a waveguide and startled by an intense burst of acoustic noise or by an air puff are capable of altering the respective startle response (Seaman et al., 1994). Effective microwave pulses had peak head-neck SAR of 15-86 kW/kg and SA of 16-86 mJ/kg for the 0.96-µs pulses used in the acoustic startle experiment and 55-113 kW/kg and 525-1056 mJ/kg for 7.82-µs pulses used in the air-puff experiment. Although SA in both experiments was at or above thresholds for auditory sensations, the role of microwave hearing or other type of intermediate stimulus was not studied.

Incidence of body movements in mice is reported to increase when the head of an animal is exposed to microwave energy at 1250 MHz as a burst of pulses or as gated CW microwaves when SA is 0.9 kJ/kg or greater (Brown et al., 1994). The authors suggest that subcutaneous

heating near the nose, estimated at $1.2~^{\circ}\text{C}$ and $0.24~^{\circ}\text{C/s}$ at the threshold SA, might be responsible for the effect.

4.0 MICROWAVE CONVULSIONS AND STUNNING

Three papers in the open literature report the induction of convulsions, stunning, and/or hypoactivity due to a single pulse of microwave energy to the head. Values of SA are estimated here from reported temperature changes by using the relationship SA = dTc, where SA is the specific absorption (in J/kg), dT is the temperature change (in $^{\circ}C$), and c is the approximate specific heat capacity (3700 J/kg- $^{\circ}C$).

Mice become "hypokinetic" following their exposure to less than lethal pulse energies at 2450 MHz in a microwave fixation apparatus and begin to recover within 5 min after exposure (Modak et al., 1981). The elevations of brain temperature are 2 and 4 $^{\circ}$ C for pulses 15 and 25 ms long, respectively. The pulse energies are 0.11 and 0.18 kJ and the reported temperature changes give estimated head SA as 7.4 and 14.8 kJ/kg. The 25-ms pulse causes a significant reduction in brain acetylcholine content.

A similar observation also comes from another laboratory using a microwave fixation device for exposure. Rats with heads exposed to a 220-ms pulse of microwave energy at 2450 MHz appear "stunned and hypoactive" for 5-10 s after exposure (Miller et al., 1987). The increase in brain temperature is 3.1 °C for estimated pulse energy of 0.77 kJ and estimated SA of 11.5 kJ/kg.

A specially designed circular waveguide is used in a third study. Rats with heads exposed to a 50-360-ms pulse of microwave energy at 915 MHz were "stunned" when brain SA was 28 kJ/kg or greater (Guy & Chou, 1982). The corresponding temperature increase in the brain was about 8 °C. The reaction consisted of seizures for about a minute after exposure followed by an "unconscious state" lasting 4-5 min.

5.0 MICROWAVE FIXATION

Microwave fixation is the process in which microwave energy is delivered to the head of an experimental animal to "fix" tissues, specifically brain tissue for neurochemical studies. Heating of the tissue by the single application of microwave energy denatures enzymes and essentially stops chemical reactions as well as being lethal to the animal. The more rapid the heating, the more representative is the chemistry of the brain to that of the animal just before fixation.

Although the emphasis of most microwave fixation papers is on the neurochemical results, the literature reveals a small number of papers that provide sufficient information to estimate pulse energy and specific absorption. Values of SA are estimated from a reported temperature change by using the relationship SA = dTc, where SA is the specific absorption (in J/kg), dT is the temperature change (in C), and C is the approximate specific heat capacity (3700 J/kg-C).

The range of reported effective pulse energies for rats and mice is 1-12.5 kJ and the associated range of estimated SA is 72-198 kJ/kg (Butcher & Butcher, 1976; Delaney & Geiger, 1996; Medina & Stavinoha, 1977; Moroji et al., 1977; Schneider et al., 1982; Merritt et al., 1975; Stavinoha et al., 1977). The range of pulse durations in these studies is 0.15-1.5 s. The ranges of applied energy and SA agree with the respective ranges of 2-10 kJ and 57-203 kJ/kg in a review that included microwave fixation (Lin, 1989, p 169f).

6.0 STUDIES WITH TEMPO AND RELATED DEVICES

A number of studies have been performed with TEMPO (Transformer Energized Megavolt Pulsed Output) devices developed to radiate high voltage pulses with durations of 20-80 ns. TEMPO pulses can be represented in the frequency domain by a band of frequencies more broad than pulse-modulated microwaves. TEMPO pulses are often described by the center frequency (CF) of this band.

Several studies have examined behavior of rats during and after exposure to TEMPO pulses. In one of the earliest of the TEMPO studies, rats were exposed to a single 85-ns pulse with 1.3 GHz CF and estimated incident power density of 0.75-0.99 kW/cm² (Klauenberg et al., 1988). Taking the product of the power density and duration gives an estimated mean fluence of 74 μ J/cm². Using 0.2 W/kg per mW/cm² incident power density as being representative for the rats used in this study (Durney et al., 1986 (Fig. 6.16)), the peak whole-body SAR was about 0.17 kW/kg on average. Delivered in 85 ns, this corresponds to 15 μ J/kg whole-body SA per pulse. Exposure to a single pulse caused a startle-like response in some animals. In the same study, exposure to 10 such pulses at 1 Hz led to alteration in baseline activity and disruption of balance on a rotarod device.

In another early study, rats were exposed to a 140-ns pulse with 1.94 GHz CF produced by a "Gypsy" virtual cathode oscillator (vircator) (Cordts et al., 1988). A single pulse of 2.2 ± 0.9 kW/cm² (mean±SD) reduced drinking in a thirst satiation task. However, single pulses averaging 4.1 and 8.2 kW/cm² did not affect this behavior nor did any of the three power densities affect performance on a single avoidance task. Balance on a rotarod was also not changed by any pulse, but many animals were noted to "flinch" with delivery of a pulse. The product of the effective power density and pulse duration gives an estimated mean fluence of 308 μ J/cm². Taking 0.35 W/kg per mW/cm² incident power density as being representative for the 200-250 g rats used in this study (Durney et al., 1986 (Fig. 6.15 and 6.16)), the peak whole-body SAR was about 0.77 kW/kg on average. The SA of the effective 140-ns pulse is estimated to have been 108 μ J/kg.

In another TEMPO study of rats, exposure to pulses with 85-ns duration and 2.11 GHz CF caused no change in subsequent performance in an operant task or in tests of avoidance behavior (Hjeresen et al., 1989). Up to 50 pulses with peak power density averaging 10.8 kW/cm^2 were delivered over 5 min. Using 0.3 W/kg per mW/cm² incident power density as being representative for the 200-g rats used in this study (Durney et al., 1986 (Figs. 6.15 and 6.16)), the peak whole-body SAR in these experiments is estimated as 3.2 kW/kg and the corresponding whole-body peak SA as 278 µJ/kg, respectively. In a fourth reported study of rats, exposure to

pulses with 200 80-ns duration and 3.0 GHz CF over about 25 min altered performance in a subsequent time discrimination task (Raslear et al., 1993). Using data provided in the report, each pulse produced peak whole-body SAR of 7.2 MW/kg and SA of 0.58 J/kg, with similar respective values in the brain. The authors refer to an acoustic stimulus that was coincident with each TEMPO pulse.

Two studies have been reported exposing monkeys to TEMPO pulses. Exposure of the head to 27-51 pulses with 93-ns duration and 2.37 GHz CF during 20 min of a vigilance task involving discrimination of acoustic tone frequency did not change performance (D'Andrea et al., 1989). The pulses, delivered during acoustic signals used in the task, had peak power of 7-11 kW/cm². The authors estimated that each pulse produced peak whole-body SAR of 0.58-0.94 MW/kg and SA of 54-87 mJ/kg. In another study, a similar behavioral test with exposure to pulses with 20-60-ns duration and 3.0 GHz CF also showed no change in performance (D'Andrea et al., 1993). Pulses, delivered every 7.5 s and having a peak power density of 45.6 kW/cm² (about 1.8 mJ/cm² fluence), each produced approximate peak whole-body SAR of 2.21 MW/kg and SA of 1.3 J/kg. Steps were taken in these two studies to reduce the acoustic signal accompanying each TEMPO pulse.

One TEMPO study examined the effect of the pulses on rat cardiovascular parameters (Jauchem & Frei, 1995). Aortic cannulas inserted prior to exposure were used to determine heart rate and blood pressure during exposure to 10 TEMPO pulses. Two types of pulses were used in separate experiments: pulses with 40-85-ns duration, 1.7-1.8 GHz CF, and 4.5 kW/cm² mean estimated peak power density and pulses with 40-70-ns duration, 1.2-1.4 GHz CF, and 32.6 kW/cm² mean estimated peak power density. No significant change in heart rate was seen in the experiment using pulses with 1.7-1.8 GHz CF. A significant transient increase in blood pressure seen with the first one or two pulses was not present after attenuation of the sound produced by the TEMPO source. No significant change was seen in either measured variable in the experiment using pulses with 1.2-1.4 GHz CF, in which TEMPO-generated sound was attenuated for all exposures. Using 0.18 and 0.3 W/kg per mW/cm² incident power density for a medium rat of 320 g at the respective frequencies (Durney et al., 1986 (Fig. 6.16)), the peak whole-body SAR in these experiments is estimated as 0.8 and 9.8 MW/kg for the respective experiments. Using representative pulse durations of 65 and 55 ns gives corresponding whole-body peak SA as 53 and 540 mJ/kg, respectively.

One observation is common to several TEMPO studies and the "Gypsy" vircator study. The sound coincident with pulse generation was audible to investigators and had the potential to affect animal behavior. This sound seems a plausible explanation for the sudden animal movement at pulse generation seen in a number of these studies. This possibility should lead to studies that take the sound of pulse generation into account and control for it. Another observation is that although a number of effects have been found in some studies, no effect was seen in other studies covering the same range of SA. This difference could be due to a number of factors including different sources, experimental protocols, and endpoints.

7.0 MICROWAVE PULSES STUDIED USING ISOLATED TISSUES

Studies that expose isolated tissue samples and cellular preparations have the potential to reveal effects at the tissue and cell level in the absence of confounding interactions with other tissues or cell types. Only a few studies have exposed isolated tissues and cellular preparations to microwave pulses with deposited energy of the magnitude that might be expected with high power microwave pulses. Coupling of microwave pulses to small preparations has been successfully accomplished by taking advantage of the shorter wavelength at 9.2-9.3 GHz and utilizing customized techniques (Pakhomov et al., 2000, 2003a).

Application of pulses delivered at regular repetition frequencies in different preparations resulted in changes that were quite similar to those seen by equivalent heating. This was the case for the decrease in inter-beat interval of isolated beating frog heart slices when exposed to 1-µs microwave pulses at 10-100 Hz repetition frequency that gave peak SAR of 250-350 MW/kg and peak SA of 250-350 J/kg for 1-10 s (Pakhomov et al., 2000). The decrease in inter-beat intervals was duplicated by lower power microwave pulses delivered with the same time average power and consequently producing the same heating. In another type of experiment, a slice of rat hippocampus was exposed to microwave pulses with 0.5-2-µs duration and 0.5-10 Hz repetition frequency that gave peak SAR up to 500 MW/kg and peak SA up to 250-1000 J/kg for 2 min (Pakhomov et al., 2003a). The evoked population spike used as an index of synaptic response was changed by microwave exposure in the same way as long as microwave pulse duration and repetition frequency were set to give the same average power, which gave the same heating. The time course of change was also consistent with the change of temperature in the preparation. Long-term potentiation of the evoked population spike, a standard test in this preparation, was also studied and it, too, showed changes consistent with a thermal basis.

Studies of the effects of single microwave pulses on the hippocampal brain slice were also performed using this exposure system (Doyle et al., 2003, 2006; Pakhomov et al., 2003b). Peak SAR and pulse SA were similar to those in the previous hippocampal work: 740 MW/kg and 1480 J/kg, respectively. Single microwave pulses were delivered at four different times relative to the electrical stimulus that evoked the population spike in the preparation. Sham exposures and exposures with unsynchronized microwave pulses were also performed. The population spike was depressed when the microwave pulse was delivered 1 ms after the electrical stimulus but not for other times tested. Because the temperature change resulting from a pulse was 0.3-0.4 °C regardless of pulse timing, the spike depression specific to the one time interval seems to be unrelated to gross temperature of the preparation.

8.0 SUMMARY

Table 1 summarizes effects observed in the various studies reviewed here. Results indicating no effect have not been placed in the table. Entries in the table are arranged in ascending order of specific absorption (SA), a standard measure of energy absorption in tissue. The SA range extends over several orders of magnitude, from the small energy deposited by a single microwave pulse to the energy delivered during an exposure up to 1.5 s long to fix tissue

for neurochemical studies. The ordering by SA is done for convenience in making comparisons and does not imply a common mechanism for the observed effects.

Documented effects occurring at the lowest specific absorptions in the µJ/kg to mJ/kg range have to do primarily with sensory stimulation. Microwave hearing is the result of the lowest pulse energy and an undisputed effect of pulsed microwaves. Note that values in the table are thresholds for the auditory sensation. Energies above the thresholds also lead to sensation, but extensive exploration of the sensation is not reported in the literature. The modification of the reflexive startle response in rats by microwave pulses occurred for SA values of tens to hundreds of mJ/kg. The modification could have been due to an auditory stimulation or stimulation of another sensory modality, such as due to instantaneous heating of skin. The same effect can be expected at higher SA values and, possibly, at lower SA values that are above the undetermined threshold for the effect. The startle-like response seen at small SA for TEMPO pulses could possibly also be due to similar stimulation.

At somewhat higher SA values, behavior changed in rats exposed to 200 TEMPO pulses, with pulse SA estimated to be 0.58 J/kg. The basis for this effect on behavior has not been reported.

Effects at the highest SA values in the table have been obtained using customized apparatus used to expose, with one exception, the head of a rodent to microwave pulses. The startle-like reaction seen at 0.9 kJ/kg-pulse SA was attributed to heating of the nose area of the test subject, but the sound of pulse generation might have contributed to an overall effective stimulus. The report of change in neural signals in the isolated hippocampal slice at 1.48 J/kg is significant because it illustrates the primary importance of pulse timing and the lesser importance of heating of the tissue, at least in the preparation tested.

The last two entries in the table are examples of what sufficiently high energy levels delivered to the head can do. The high SA values are achieved by means of longer exposure durations and special apparatus. Values of SA in the 7-28 kJ/kg range have been shown to stun or incapacitate animals without being lethal. On the other hand, exposures with SA values of 72-198 kJ/kg are used to kill the animal for study of brain neurochemistry. Between 28 and 72 kJ/kg SA deposited in the head one can expect a range of effects from stun through death. The 28-kJ/kg value was a threshold for stunning in the study in which it was determined, but, otherwise, the effects in the 28-72 kJ/kg range have not been reported.

Overall, the biological effects of pulsed microwaves range from weak sensory stimulation to death in the laboratory, as summarized in Table 1. Energy deposited in biological tissue by a pulse or other brief application serves as a basis for comparison of the different types of biological effects.

Table 1: Biological effects of high peak power microwave pulses reported in the open literature

Effect	Fluence	Specific Absorption	Notes
Microwave	1.5-1240 μJ/cm ²	0.6-180 mJ/kg Animal	
Hearing Threshold	2.3-40 μJ/cm ²	N/A	Human
Startle-like reaction, Disruption of Activity	74 μJ/cm ² E 308 μJ/cm ² E	15 μJ/kg E 108 μJ/kg E	TEMPO, Rodent 10 pulses for change in activity, Possible sound with pulse
Startle Modification	N/A	16-86 mJ/kg, 525-1056 mJ/kg	Rodent head
Change in Post-Exposure Behavior	12.8 mJ/cm ²	0.58 J/kg	TEMPO, Rodent 200 pulses at 0.125 Hz Sound with pulse
Startle-like Motion Threshold	N/A	0.9 kJ/kg	Rodent head
Synaptic Modification	N/A	1.48 kJ/kg	Hippocampal slice Different effects for many pulses delivered in 1-120 s
Stunning or Hypoactivity Up to 5 minutes	N/A	7.4-14.8 kJ/kg E 11.5 kJ/kg E ≥28 kJ/kg	Rodent head 0.010-0.36 s 3 different reports
Brain fixation, Death	N/A	72-198 kJ/kg E	Rodent head 0.15-1.5 s several reports

Results are for a single pulse unless otherwise noted. E indicates that an estimate was made based on data in the original report. N/A = not an applicable measure for this effect.

REFERENCES

- Al-Saadi, M. H., Nadeau, V., & Dickinson, M. R. (2006). A novel modeling and experimental technique to predict and measure tissue temperature during CO₂ laser stimuli for human pain studies. *Lasers Med Sci*, 21, 95-100.
- Arendt-Nielsen, L. & Bjerring, P. (1988). Sensory and pain threshold characteristics to laser stimuli. *J Neurol Neurosurg Psychiatry*, 51, 35-42.
- Arendt-Nielsen, L. & Chen, A. C. (2003). Lasers and other thermal stimulators for activation of skin nociceptors in humans. *Neurophysiol Clin*, *33*, 259-268.
- Blick, D. W., Adair, E. R., Hurt, W. D., Sherry, C. J., Walters, T. J., & Merritt, J. H. (1997). Thresholds of microwave-evoked warmth sensations in human skin. *Bioelectromagnetics*, 18, 403-409.
- Brown, D. O., Lu, S. T., & Elson, E. C. (1994). Characteristics of microwave evoked body movements in mice. *Bioelectromagnetics*, 15, 143-161.
- Butcher, L. L. & Butcher, S. H. (1976). Brain temperature and enzyme histochemistry after high intensity microwave irradiation. *Life Sci*, 19, 1079-1087.
- Chéry-Croze, S. (1983). Painful sensation induced by a thermal cutaneous stimulus. *Pain*, 17, 109-137.
- Chou, C. K., Guy, A. W., & Galambos, R. (1982). Auditory perception of radio-frequency electromagnetic fields. *J Acoust Soc Am*, 71(6), 1321-1334.
- Cook, H. F. (1952). The pain threshold for microwave and infra-red radiations. *J Physiol*, 118, 1-11.
- Cordts, R. E., Merritt, J. H., Erwin, D. N., Hardy, K. A., & Yochmowitz, M. G. (1988). Behavioral response of rats exposed to high-power microwave radiation (Technical Report USAFSAM-TR-87-30). Brooks Air Force Base: USAF School of Aerospace Medicine.
- D'Andrea, J. A., Knepton, J., Cobb, B. L., Klauenberg, B. J., Merritt, J. H., & Erwin, D. N. (1989). *High peak power microwave pulses at 2.37 GHz: No effect on vigilance performance in monkeys* (Joint Naval Aerospace Medical Research Laboratory Research Report, NAMRL-1348, and USAF School of Aerospace Medicine, USAFSAM-TR-89-21).
- D'Andrea, J. A., Cobb, B. L., Knepton, J., & Bates, F. (1993). *Behavioral performance in monkeys exposed to Tempo high-peak-power microwave pulses at 3 GHz* (NAMRL-1389). Naval Aerospace Medical Research Laboratory.

- Defrin, R., Givon, R., Raz, N., & Urca, G. (2006). Spatial summation and spatial discrimination of pain sensation. *Pain*, *126*, 123-131.
- Delaney, S. M. & Geiger, J. D. (1996). Brain regional levels of adenosine and adenosine nucleotides in rats killed by high-energy focused microwave irradiation. *J Neurosci Methods*, 64, 151-156.
- Doyle, J., Murphy, M. R., & Pakhomov, A. G. (2003). High-power microwave pulses phased with evoked synaptic potentials may affect synaptic transmission [Abstract]. *25th Annual Meeting of the Bioelectromagnetics Society*, 359.
- Doyle, J., Stuck, B., Murphy, M. R., & Pakhomov, A. G. (2006). Suppression of synaptic transmission in hippocampus by extremely-high power microwave pulses synchronized with neuronal excitation. In S. N. Ayrapetyan & M. S. Markov (Eds.), *Bioelectromagnetics Current Concepts* (pp. 123-133). Amsterdam: Springer.
- Durney, C. H., Massoudi, H., & Iskander, M. F. (1986). *Radiofrequency radiation dosimetry handbook, Fourth Edition* (Technical Report USAFSAM-TR-85-73). Brooks Air Force Base: USAF School of Aerospace Medicine.
- Eijkman, E. & Vendrik, A. J. (1961). Dynamic behavior of the warmth sense organ. *J Exp Psychol*, 62, 403-408.
- Elder, J. A. & Chou, C. K. (2003). Auditory response to pulsed radiofrequency energy. *Bioelectromagnetics Suppl*, *6*, S162-S173.
- Frey, A. H. (1961). Auditory system response to radio frequency energy. *Aerospace Med*, 32, 1140-1142.
- Frey, A. H. (1962). Human auditory system response to modulated electromagnetic energy. *J Appl Physiol*, 17, 689-692.
- Frey, A. H. (1967). Brain stem evoked responses associated with low-intensity pulsed UHF energy. *J Appl Physiol*, 23, 984-988.
- Gardner, E. P., Martin, J. H., & Jessell, T. M. (2000). The Bodily Senses. Chapter 22. In E. R. Kandel, J. H. Schwartz, & T. M. Jessell, (Eds.), *Principles of Neural Science* (4th ed., pp. 430–449). New York: McGraw-Hill.
- Graven-Nielsen, T., Arendt-Nielsen, L., & Mense, S. (2002). Thermosensitivity of muscle: high-intensity thermal stimulation of muscle tissue induces muscle pain in humans. *J Physiol*, 540, 647-656.

- Guy, A. W., Chou, C. K., Lin, J. C., & Christensen, D. (1975). Microwave-induced acoustic effects in mammalian auditory systems and physical materials. *Ann N Y Acad Sci*, 247, 194-218.
- Guy, A. W. & Chou, C. K. (1982). Effects of high-intensity microwave pulse exposure of rat brain. *Radio Science*, 17(5S), 169S-178S.
- Hendler, E. (1968). Cutaneous receptor response to microwave irradiation. In J. D. Hardy (Ed.), *Thermal Problems in Aerospace Medicine* (pp. 149–161). Surry: Unwin Bros Ltd., Surrey.
- Hendler, E., Hardy, J. D., & Murgatroyd, D. (1963). Skin heating and temperature sensation produced by infrared and microwave irradiation. In C. M. Herzfeld & J. D. Hardy (Eds.), *Temperature: Its Measurement and Control in Science and Industry* (Vol 3, Part 3, pp. 211-230). New York: Reinhold.
- Heynick, L. N. (2003, November). A review of the biological effects of pulsed radiofrequency electromagnetic fields (RFEMF). Report submitted to Advanced Information Engineering Services, A General Dynamics Company.
- Hilz, M. J., Stemper, B., Axelrod, F. B., Kolodny, E. H., & Neundörfer, B. (1999). Quantitative thermal perception testing in adults. *J Clin Neurophysiol*, *16*, 462-471.
- Hjeresen, D. L., Umbarger, K. O., Klauenberg, B. J., & Erwin, D. N. (1989). Lack of behavioral effects of high-peak-power microwave pulses from an axially extracted virtual cathode oscillator (Technical Report USAFSAM-TR-89-24). Brooks Air Force Base: USAF School of Aerospace Medicine.
- Hynynen, K. & DeYoung, D. (1988). Temperature elevation at muscle-bone interface during scanned, focused ultrasound hyperthermia. *Int J Hyperthermia*, *4*, 267-279.
- Jauchem, J. R. & Frei, M. R. (1995). High-peak-power microwave pulses: Effects on heart rate and blood pressure in unanesthetized rats. *Aviat Space Environ Med*, 66, 992-997.
- Johnson, C. C. & Guy, A. W. (1972). Nonionizing electromagnetic wave effects in biological materials and systems. *Proc IEEE*, 60, 692-718.
- Jørum, E., Warncke, T., & Stubhaug, A. (2003). Cold allodynia and hyperalgesia in neuropathic pain: the effect of N-methyl-D-aspartate (NMDA) receptor antagonist ketamine--a double-blind, cross-over comparison with alfentanil and placebo. *Pain*, *101*, 229-235.
- Justesen, D. R., Adair, E. R., Stevens, J. C., & Bruce-Wolfe, V. (1982). A comparative study of human sensory thresholds: 2450-MHz microwaves vs far-infrared radiation. *Bioelectromagnetics*, *3*, 117-125.

- Klauenberg, B. J., Merritt, J. H., & Erwin, D. N. (1988). *Behavioral effects of exposure to the Tempo high-power microwave system* (Technical Report USAFSAM-TR-87-35). Brooks Air Force Base: USAF School of Aerospace Medicine.
- Lin, J. C. (1978). *Microwave Auditory Effects and Applications*. Springfield, IL: Charles C. Thomas.
- Lin, J. C. (1986). Computer methods of field intensity predictions. In C.P. Polk & E. Postow (Eds.), *CRC Handbook of Biological Effects of Electromagnetic Fields* (pp. 273-313). Boca Raton, Florida: CRC Press.
- Lin, J. C. (1989). Pulsed radiofrequency field effects in biological tissues. In J. C. Lin (Ed.), *Electromagnetic Interactions with Biological Systems* (pp. 165-177). New York: Plenum Press.
- Lin, J. C. (1990). Auditory perception of pulsed microwave radiation. Chapter 12. In O. P. Gandhi (Ed.), *Biological Effects and Medical Applications of Electromagnetic Energy* (pp. 277-318). Englewood Cliffs, NJ: Prentice Hall.
- Lin, J. C. & Wang, Z. (2006). High-field MRI microwave pulses induce sound pressure waves in human heads. *Annual Meeting of the Bioelectromagnetics Society*, Abstracts pp. 408-409.
- Lu, S. T. & de Lorge, J. O. (2000). Biological effects of high peak power radiofrequency pulses. In J. C. Lin (Ed.), *Advances in Electromagnetic Fields in Living Systems* (Vol. 3, pp. 207-264). New York: Plenum Press.
- Lumpkin, E. A. & Caterina, M. J. (2007). Mechanisms of sensory transduction in the skin. *Nature*, 445, 858-865.
- McAfee, R. D. (1961). Neurophysiological effect of 3-cm microwave radiation. *Am J Physiol*, 200, 192-194.
- McAfee, R. D. (1962). Physiological effects of thermode and microwave stimulation of peripheral nerves. *Am J Physiol*, 203, 374-378.
- Medina, M. A. & Stavinoha, W. B. (1977). Labile intermediary metabolites in rat brain determined after tissue inactivation with microwave irradiation. *Brain Res*, *132*, 149-152.
- Merritt, J. H., Medina, M. A., & Frazer, J. W. (1975). Neurotransmitter content of mouse brain after inactivation by microwave heating. *Res Commun Chem Pathol Pharmacol*, 10, 751-754.
- Meyer, J. L. (1984). The clinical efficacy of localized hyperthermia. *Cancer Res*, 44 (Suppl), 4745S-4751S.

- Miller, D. B., Blackman, C. F., & O'Callaghan, J. P. (1987). An increase in glial fibrillary acidic protein follows brain hyperthermia in rats. *Brain Res*, *415*, 371-374.
- Modak, A. T., Stavinoha, W. B., & Deam, A. P. (1981). Effect of short electromagnetic pulses on brain acetylcholine content and spontaneous motor activity of mice. *Bioelectromagnetics*, 2(1), 89-92.
- Moroji, T., Takahashi, K., Ogura, K, Toishi, T., & Arai, S. (1977). Rapid microwave fixation of rat brain. *J Microw Power*, *12*, 273-286.
- Nahra, H. & Plaghki, L. (2005). Innocuous skin cooling modulates perception and neurophysiological correlates of brief CO₂ laser stimuli in humans. *Eur J Pain*, 9, 521-530.
- Nielsen, J. & Arendt-Nielsen, L. (1998). The influence of rate of temperature change and peak stimulus duration on pain intensity and quality. *Somatosens Mot Res*, 15, 220-229.
- Ørstavik, K., Norheim, I., & Jørum, E. (2006). Pain and small-fiber neuropathy in patients with hypothyroidism. *Neurology*, 67, 786-791.
- Pakhomov, A. G., Mathur, S. P., Doyle, J., Stuck, B. E., Kiel, J. L., & Murphy, M. R. (2000). Comparative effects of extremely high power microwave pulses and a brief CW irradiation on pacemaker function in isolated frog heart slices. *Bioelectromagnetics*, 21, 245-254.
- Pakhomov, A. G. & Murphy, M. R. (2000). A comprehensive review of the research on biological effects of pulsed radiofrequency radiation in Russia and the former Soviet Union. In J. C. Lin (Ed.), *Advances in electromagnetic fields in living systems* (Vol. 3, pp. 265-290). New York: Plenum Press.
- Pakhomov, A. G., Doyle, J., Stuck, B. E., & Murphy, M. R. (2003a). Effects of high power microwave pulses on synaptic transmission and long term potentiation in hippocampus. *Bioelectromagnetics*, 24, 174-181.
- Pakhomov, A. G., Doyle, J., & Murphy, M. R. (2003b). Alteration of synaptic transmission by neuron excitation-synchronized high-power microwave pulses: a replication study [Abstract]. EBEA 2003, 6th International Congress of the European Bioelectromagnetics Association, 110.
- Pedersen, J., Reddy, H., Funch-Jensen, P., Arendt-Nielsen, L., Gregersen, H., & Drewes, A. M. (2004a). Cold and heat pain assessment of the human esophagus after experimental sensitization with acid. *Pain*, 110, 393-399.
- Pedersen, J., Reddy, H., Funch-Jensen, P., Arendt-Nielsen, L., Gregersen, H., & Drewes, A. M. (2004b). Differences between male and female responses to painful thermal and mechanical stimulation of the human esophagus. *Dig Dis Sci*, 49, 1065-1074.

- Pickering, G., Jourdan, D., Eschalier, A., & Dubray, C. (2002). Impact of age, gender and cognitive functioning on pain perception. *Gerontology*, 48, 112-118.
- Raslear, T. G., Akyel, Y., Bates, F., Belt, M., & Lu, S. T. (1993). Temporal bisection in rats: the effects of high-peak-power pulsed microwave irradiation. *Bioelectromagnetics*, *14*, 459-478.
- Röschmann, P. (1991). Human auditory system response to pulsed radiofrequency energy in RF coils for magnetic resonance at 2.4 to 170 MHz. *Magn Reson Med*, 21, 197-215.
- Schaffner, N., Wittwer, A., Kut, E., Folkers, G., Benninger, D. H., & Candia, V. (2008). Heat pain threshold and tolerance show no left-right perceptual differences at complementary sites of the human forearm. *Neurosci Lett*, 440, 309-313.
- Schneider, D. R., Felt, B. T., & Goldman, H. (1982). On the use of microwave radiation energy for brain tissue fixation. *J Neurochem*, *38*, 749-752.
- Seaman, R. L., Beblo, D. A., & Raslear, T. G. (1994). Modification of acoustic and tactile startle by single microwave pulses. *Physiol Behav*, *55*, 587-595.
- Seaman, R. L. & Lebovitz, R. M. (1989). Thresholds of cat cochlear nucleus neurons to microwave pulses. *Bioelectromagnetics*, 10, 147-160.
- Shimm, D. S., Hynynen, K. H., Anhalt, D. P., Roemer, R. B., & Cassady. J. R. (1988). Scanned focussed ultrasound hyperthermia: initial clinical results. *Int J Radiat Oncol Biol Phys*, 15, 1203-1208.
- Stavinoha, W. B., Frazer, J., & Modak, A. T. (1977). Microwave fixation for the study of acetylcholine metabolism. In D. J. Jenden (Ed.), *Cholinergic Mechanisms and Psychopharmacology* (pp. 169-179). New York: Plenum Press.
- Strigo, I. A., Carli, F., & Bushnell, M. C. (2000). Effect of ambient temperature on human pain and temperature perception. *Anesthesiology*, 92, 699-707.
- Vendrik, A. J. & Vos, J. J. (1958). Comparison of the stimulation of the warmth sense organ by microwave and infrared. *J Appl Physiol*, 13, 435-444.
- Walters, T. J., Blick, D. W., Johnson, L. R., Adair, E. R., & Foster, K. R. (2000). Heating and pain sensation produced in human skin by millimeter waves: Comparison to a simple thermal model. *Health Phys*, 78, 259-267.
- Wang, Z. & Lin, J. C. (2005). RF induced thermoelastic pressure waves in different size human head models in MRI birdcage coils [Abstract]. *Annual Meeting of the Bioelectromagnetics Society, Bioelectromagnetics*, 2005, 112-113.

- Watanabe, Y., Tanaka, T., Taki, M., & Watanabe, S. (2000). FDTD analysis of microwave hearing effect. *IEEE Trans Microwave Theory Tech*, 48, 2126-2132.
- Yarnitsky, D., Simone, D. A., Dotson, R. M., Cline, M. A., & Ochoa, J. L. (1992). Single C nociceptor responses and psychophysical parameters of evoked pain: Effect of rate of rise of heat stimuli in humans. *J Physiol*, 450, 581-592.